Microrheology and structural quantification of blood clots as a diagnosis of hypercoagulability

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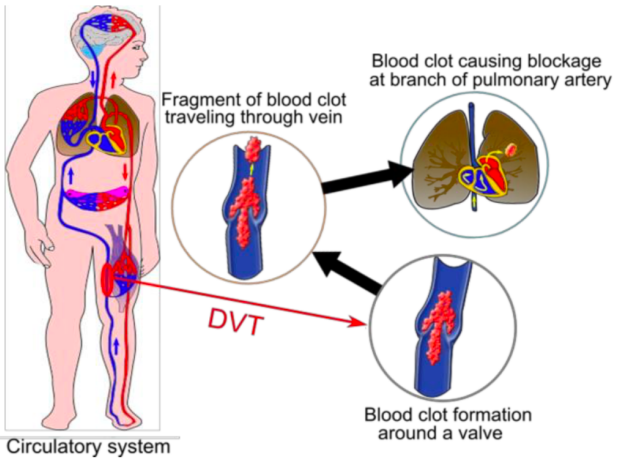
L. Wolff-Trombini et al, accepted in Biomedical Optics Express (30 june 2023)

Hypercoagulability and thrombosis

What is thrombosis?

- Clot formed in a blood vessel

- 2 types of thrombosis :
  - Arterial (major)
  - Venous



Berthomier, T. and al. *Adv. Sci. Technol. Eng. Syst. J.* **2**, 48–59 (2017).

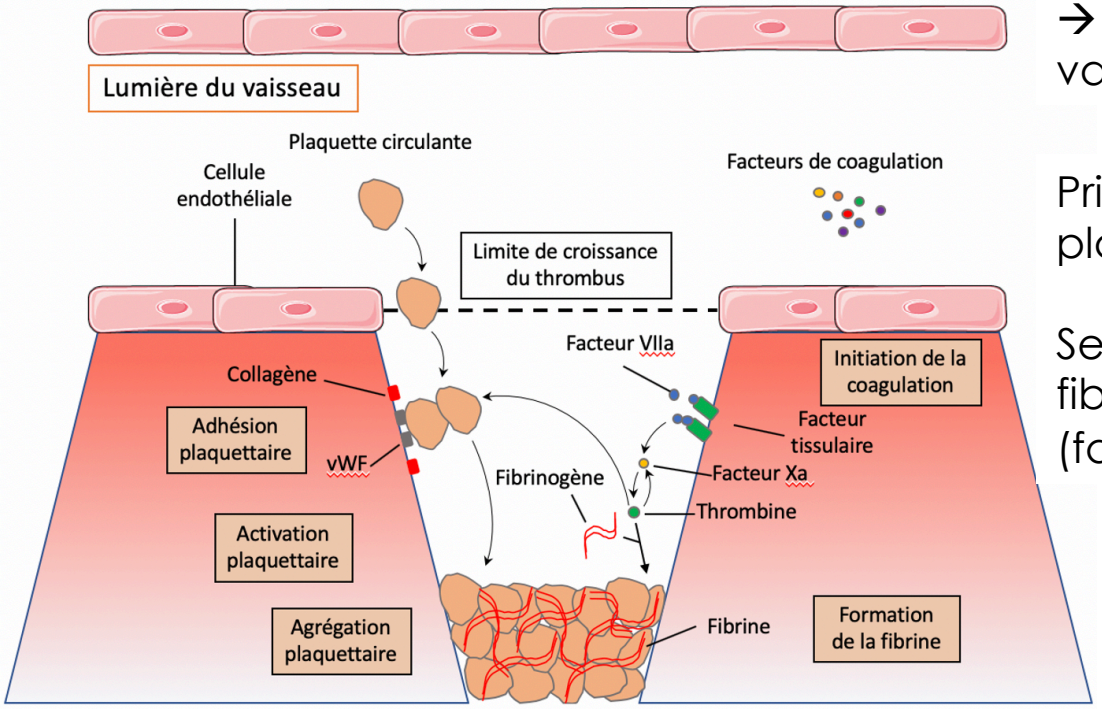
- *Complications* : recurrence and pulmonary embolism

- In France : 50 000 to 100 000 phlebitis, 40 000 P. Emb / year
- Thromboembolic events: 3rd cause of cardiovascular deaths

Problem : 50% of recurrent deep venous thrombosis events remain unexplained

Blood clot and coagulation

Physiological hemostasis and its regulation



→ Maintaining blood flow and healing vascular breaches

Primary hemostasis: agregation of platelets in the breach

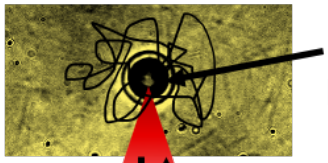
Secondary hemostasis: formation of fibrin based on coagulation factors (factors I to XII)

→ We start from poor platelet plasma (PPP) = no red or white blood cells nor platelets but with coagulation factors (non activated proteins)

→ We initiate coagulation by adding tissue factor, calcium and phospholipids + microbeads for our optical microrheology

Passive microrheology

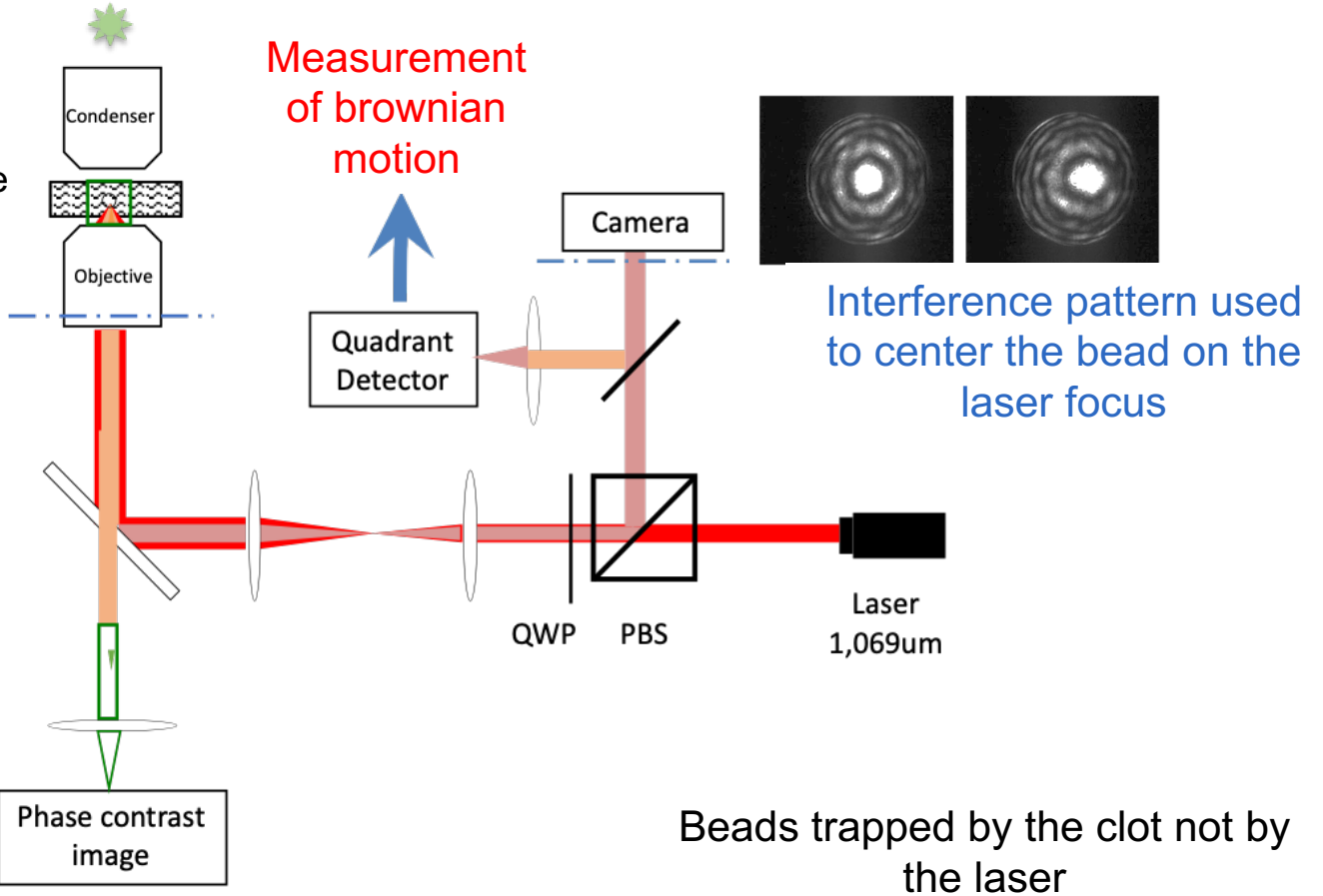
Microbeads are incorporated in the blood clot and their brownian motion under thermal fluctuations is measured using an optical tweezer setup when the reflection of a laser beam focused on the bead gives access very precisely to the position of the bead.



Blood clot with 6 µm polystyrene beads

Illumination

Détection du mouvement



Interference pattern used to center the bead on the laser focus

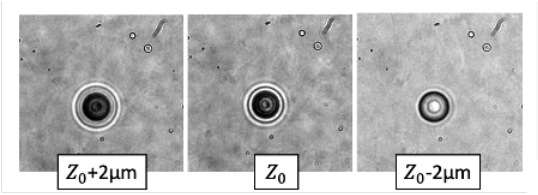


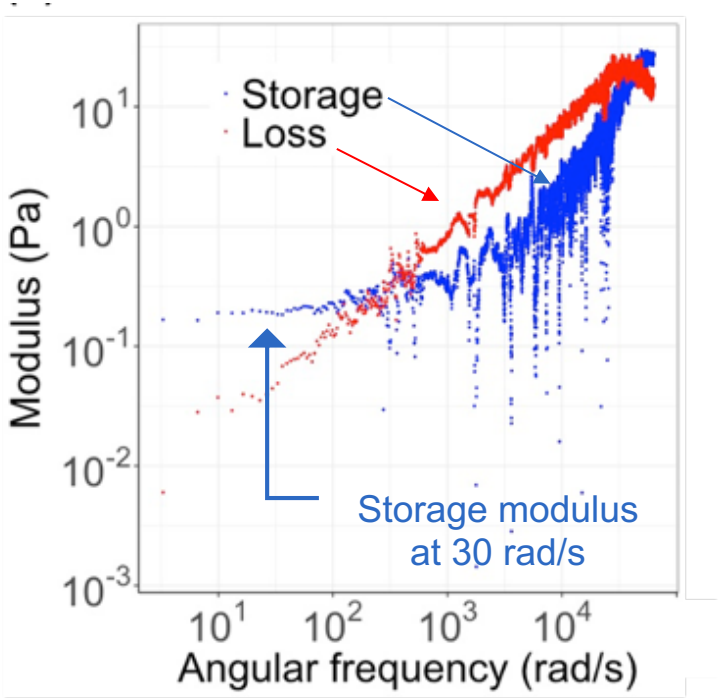
Image used to position the bead on the laser focus

Beads trapped by the clot not by the laser
=> Need to center the bead on the laser focus

Extraction of viscoelastic ppties

- Brownian motion recorded with high spatial and temporal resolution (0.1 to 10kHz)

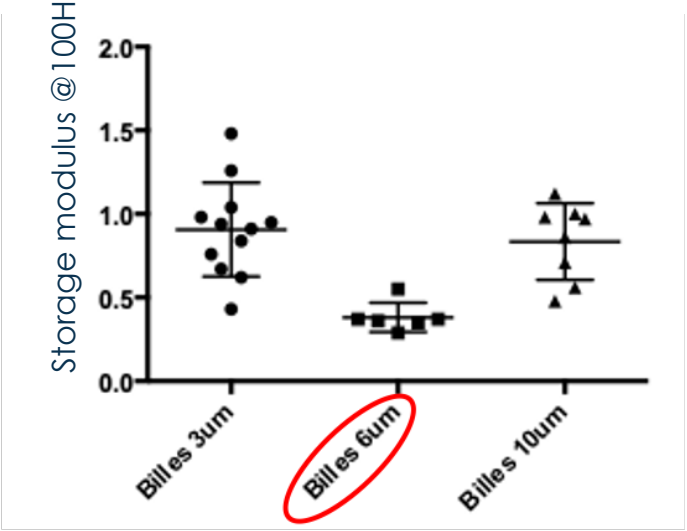
=> Local viscoelastic properties of the blood clot as a function of frequency



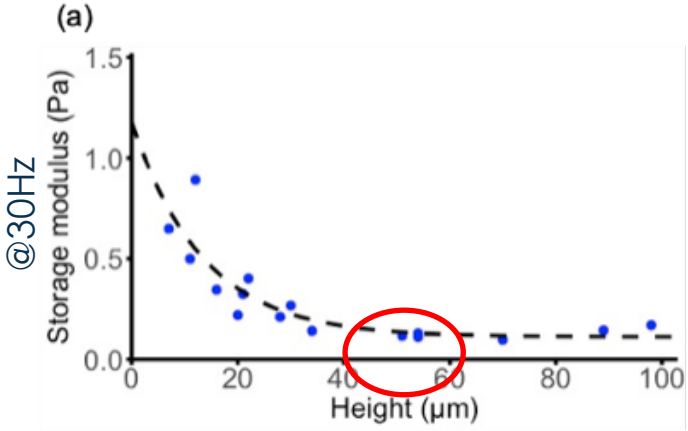
Same shape for all the curves => characterization with one measurement = storage modulus at 30 rad/s

Measurement protocol

Choice of microrheology parameters to define a reference measurement on normal clots



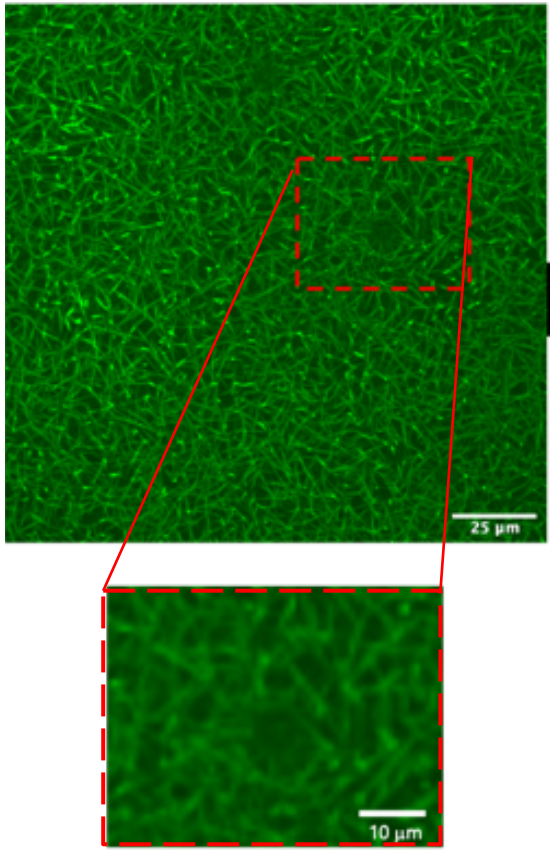
Choice of bead diameter: 6 µm
=> less dispersion in the measurement



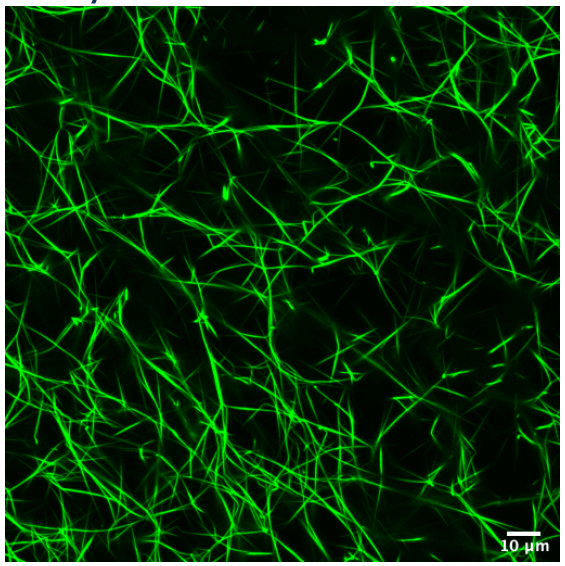
Choice of bead height
(distance to coverslip): 40 to 60µm
=> less variation with height

Confocal imaging of fibrin

Correlation of mechanical measurements with confocal images of fibrin network (fibrinogen labeled with Alexa488)



Confocal image of a fibrin network.
Control from a human pool.
FVIII=100%
Scale bar 25µm. Zoom on an area with a bead (scale bar 10µm).

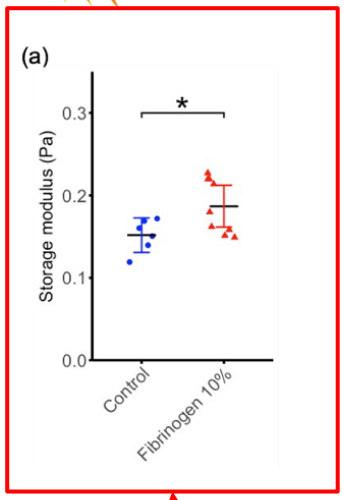


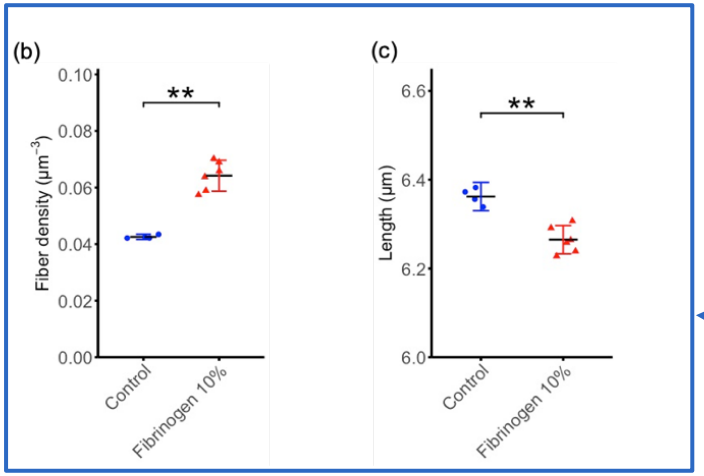
Hemophilic patient
FVIII=1,1%

Example of confocal image of a looser fibrin network

Quantification of confocal images: fiber density and length

Characterization of induced hypercoagulability

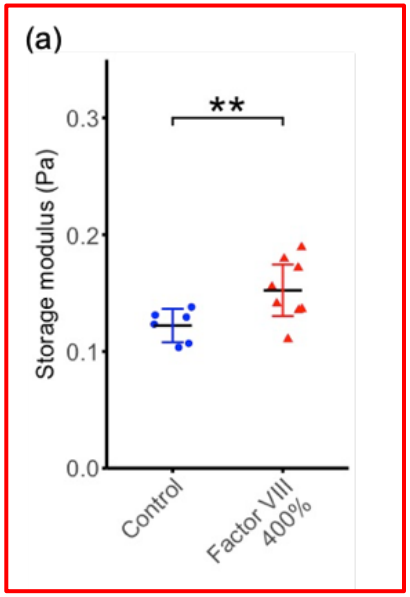


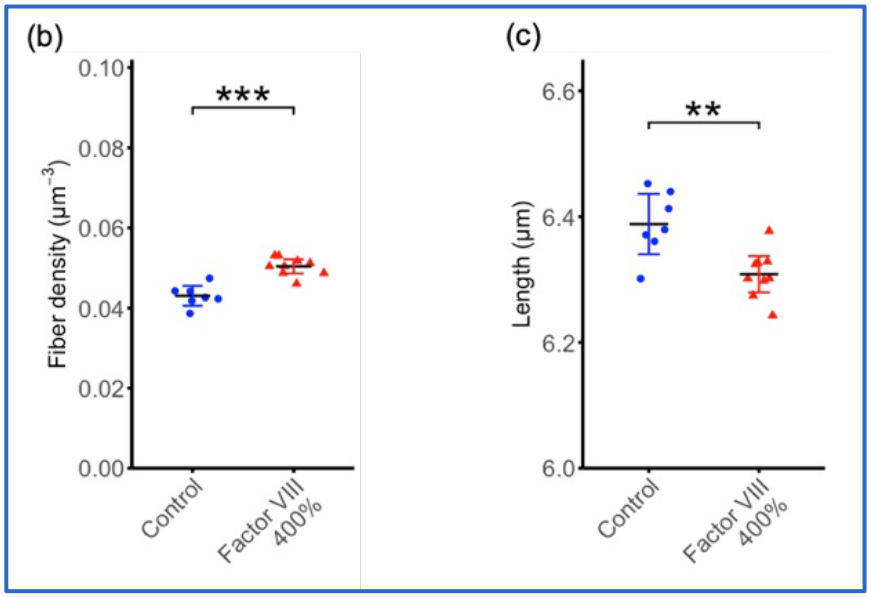


Blood clots with 10% more fibrinogen are more rigid than control clots

structural imaging

Microrheology measurements



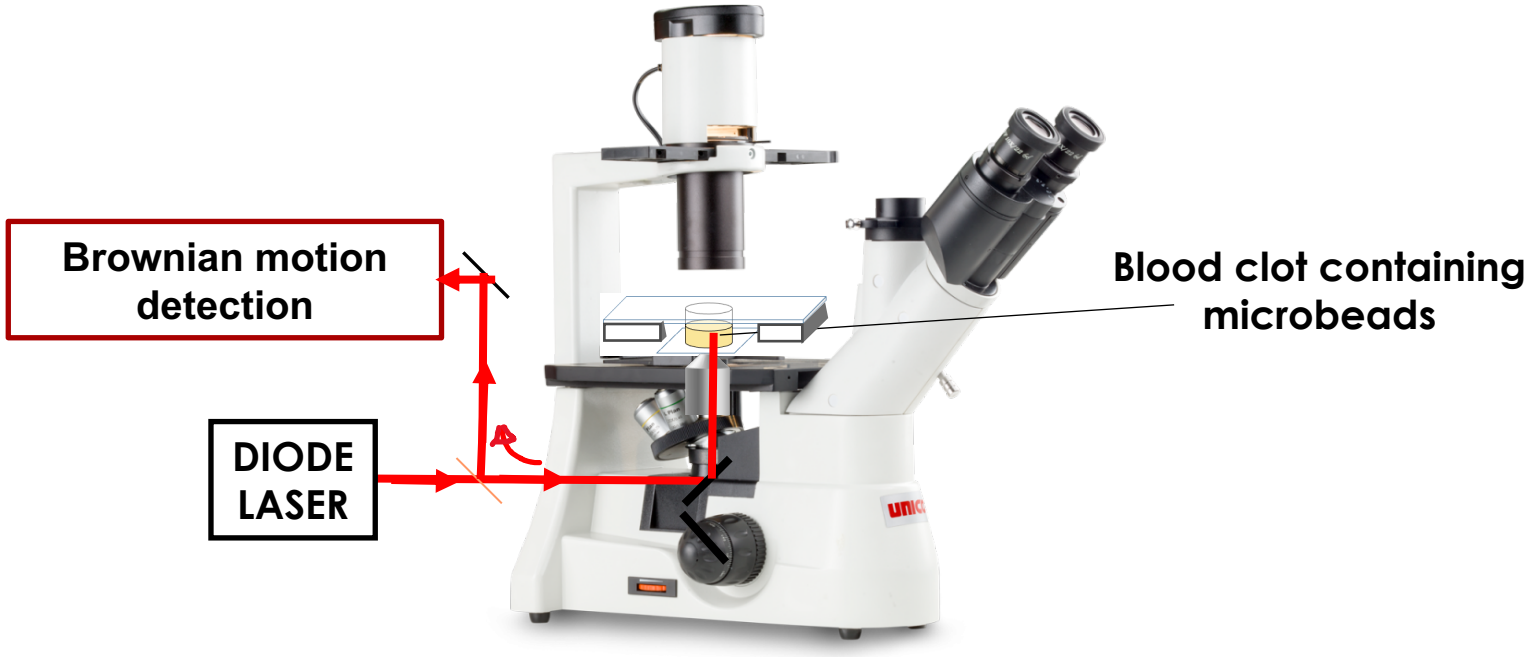


Blood clots supplemented at 400% with one specific coagulation factor (Factor VIII) are more rigid than control clots

1 point=1 clot (4 beads)

Perspectives

→ Characterize blood clots from patients with coagulation pathology (thrombotic or hemophilic)



→ Compact and automatized prototype transportable to the hospital